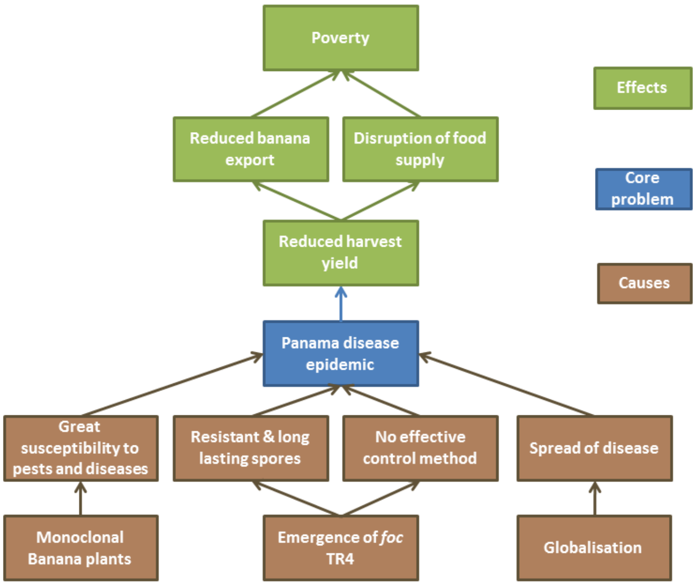
BANANA GUARD

This application scenario was developed by the iGEM team Wageningen UR 2014 in collaboration with Synenergne. With this scenario we aim to explore how our product could be applied and what effects this could have. Furthermore the potential risks of our product are considered and compared to alternatives.

The problem

Not so long ago we enjoyed a different and sometimes even considered better tasting banana than the ones we eat now, the Gros Michel. Gros Michel was wiped out over the world in the 1950's by pandemic called Panama disease pandemic in the 1950's. The banana we consume now, known as *Cavendish*, was found to be resistant against the fungus that caused the disease, *Fusarium oxysporum f.sp.cubense*. Unfortunately, the situation may repeat itself. A new form of this fungus, called Tropical Race 4 emerged at the end of the 1990's and has ravaged hectares of Cavendish banana plantations. It is only a matter of time before the disease will spread to other banana producing regions (e.g. South America) where the pathogen is not yet present.



Banana trade is an important economical factor for banana producing countries. Annually over 100 million tons of bananas with an value of five billion dollar is produced. Besides the economical factor there is the nutritional dependency on bananas. Only 15% of the global production is exported while the remaining 85% is consumed locally. Bananas are mostly cultivated in developing countries such as the Philippines and Indonesia, where it is not only an important export product but also a staple food, comparable to wheat, rice or potatoes. Thus, the Panama disease is not only a threat to the livelihood of farmers and economy of the producing countries, but a worldwide threat to food security.

Panama disease is extremely virulent due to the combination of different factors (presented in brown squares in the Picture XX). Both, commercially and non-commercially grown banana plants have very low diversity. Moreover, virtually all commercially grown banana plants are clones of each other. Due to the low diversity they are extremely susceptible to disease. Scientists fought the Panama disease with different approaches such as searching for a resistant, commercially viable cultivar and chemical treatment (with?)by fungicides. The latter was proved environmentally unfriendly and ineffective as the *Fusarium* fungus forms resistant spores. Spores can lay dormant in the soil for decades and still be effective when new bananas are planted. The combination of non-diverse plants and resistant spores leads to a very effective and rapid spread of the disease. The spread is even faster due to the increasing transit of goods all over the world, giving the fungus a chance to spread from one continent to another via important trade roods.

How does it work?

Our approach to prevent a possible banana extinction is to utilize and improve a naturally occurring bacteria called *Pseudomonas putida* to stop *Fusarium* whenever the pathogenic fungus is sensed. *P. putida* is known to reside around the roots of plants where it is beneficial to plants and inhibits *Fusarium* infection. By increasing this inhibitory effect, our bacteria will be able to prevent *Fusarium* infection more effectively and remediate infected soils.

When the bacteria are applied to the soil, either via fertilizer or irrigation, BananaGuard will colonize the plant roots and build a defence against *Fusarium*. BananaGuard will then lay dormant until detection of the fungus and will not express any fungal inhibitors, keeping the effect of our product on the soil and soil ecology as low as possible. *Fusarium* can be specifically detected by the presence of a toxin named fusaric acid, which is needed to infect the banana root system. When fusaric acid is detected, BananaGuard will secrete several naturally occurring fungal growth inhibitors and fungicides in order to inhibit the pathogen.
When *Fusarium* dies and no more fusaric acid can be detected, a regulatory system in BananaGuard will activate a kill switch to self-destruct the bacteria. This mechanism will ensure that the modified bacteria will not remain in the soil no longer than necessary. In addition, we will use a mutually dependent double plasmid system to prevent spread of BananaGuards synthetic genes. If other soil microorganisms acquire genetic material of B, a toxin coded in that genetic material will prevent spread of the host accepting the material.

Goal & Business plan

Our aim is to save the commercial growth of bananas and prevent hunger caused by extinction of the bananas with a product that can prevent and contain the Panama disease and other soil born fungi that threaten our food supply.

To achieve the aim stated above, our product needs to be low-cost, accessible, easily usable, eco-friendly and safe. The product that we developed is going to be competitive and accessible because it can be sent via mail in dried format. With an addition of water and basic source of nutrients BananaGuard can be added to existing fertilizer or irrigation systems. Since safety of genetically modified organisms is a very sensitive topic worldwide, we already introduced two safety mechanisms in the product itself (explained below). Moreover, we would like to propose educational lectures for workers at banana plantations to spread awareness about the work with GM organisms.

Context & Application

The BananaGuard application we designed was carefully considered. Aspects such as use of our product by the targeted users, the research, production costs, the deployment and regulations were thought through.

Ideally, BananaGuard would be applied as a dried ground supplement which farmers under risk of Fusarium infection could add to their soil. These supplements could then be bought form a consortium funded factory for free or at cost-price to local producers which could produce pellets of dried bacteria. These pellets can easily be stored for several weeks to months. The farmers would buy the pellets from the local suppliers or at the factory via post or internet. To reassure safe usage and proper knowledge for the use of our product free and local education will be given to (potential) buyers.

Farmers can dissolve the dried pellets and apply it to the plant soil, which could possibly be combined with the application of products such as fertilizer. Since farmers generally will already supplement their plants with fertilizer or pesticides, this would only add a small extra step to their current practices. The bacteria added to the soil would protect the plants several months to years after which the supplements will have to be reapplied, depending on the final implementation of the kill switch. The farmers can also grow the bacteria themselves with a minimal amount of materials and training, since *P. putida* is an easily grown bacteria as long as there is the possibility to work sterile. The batch of bacteria can be used to grow more bacteria a few times but then needs to be renewed to assure the quality of the product and to remove contaminations.

Although the application would be simple, regulations concerning an engineered soil bacterium for bio-control are complex and different in most countries. With the current regulations in Europe for example, application of our product would not be possible in the near future. However, our main target area, South America and Asia have far less stringent rules about the use of GMO products. It is important to note that the use of GMO's is still a hot topic in the public debate and regulations are constantly changing.

Production & Costs

One advantage that BananaGuard would have is the low production cost. Although the upfront research and testing cost would be significant, the production of the bacterial supplements itself can be low-cost. Furthermore, depending on the final specifications of BananaGuard it could give a long term resistance (a full crop cycle to several years), so repeated application will not be necessary which will also keep costs down. The only real expense is a factory where the bacteria are being made and send. This can be done relatively low-cost since the resources to grow bacteria are not expensive.

This combination of high developmental cost and low deployment costs, would be ideal to support smaller (third world) farmers who depend on the production of bananas as an important part of their food supply. Funding of the developmental costs could be a consortium of large banana producing/trading companies, NGO's and governments of the concerning countries. Finally, all preliminary work done by the iGEM team will be publicly available, allowing interested companies to study and further improve our product.

Risks

To assess the risk of our product we will have to look at several factors. Firstly, there are the risks involved in the usage of the separate parts and the system as a whole. Secondly, we need to consider the risk of the system malfunctioning and what harm the system could actually when malfunctioning. Thirdly, there are the risks of unforeseen and incalculable factors. Finally, it is important to see how the risks compare to other possible solutions of our problem.

Most of the individual parts are often used and have already been found to be safe. The new parts that will have to be isolated will be from risk-class 1 organisms, which is the class containing the least dangerous microorganisms. In conclusion, the parts on their own can be considered safe. However, possible interactions between parts will also have to be considered. Although this can be difficult to predict, the fact that the parts can be considered safe and originate from nature can be taken as a good indication that no potential harmful combinations will arise.

If our system would malfunction there would be two major risks, firstly, nonspecific overproduction of antifungal substances and secondly the spread of modified genetic material in the environment. Because of our kill switch system, any instabilities which could lead to nonspecific overproduction of the antifungal compounds is also likely to activate the kill switch system, thereby decreasing the risk of a malfunctioning bacteria. In addition, if antifungal compounds would be overproduced, our bacteria will probably not be able to compete for growth because of the metabolic stress, although it could (temporarily) affect the local microbiome. The spread of modified genetic material will be largely prevented by the double dependent plasmid system. In the case of a series of mutations disabling this system the genetic material could still be spread. The uncontrolled spread of synthetic genes makes it very difficult to control the population with synthetic genes posing a potential risk. However, most of our actual products such as the antifungals are isolated from soil bacteria. Related genes can thus already be found in the soil microbiome and as so can be considered a limited risk even when our protection fails.

Biological systems are by nature subject to noise and change over time. Lots of interactions and principles are still unknown. Although lots of interactions can be predicted, tested or calculated there always remains the unpredictable. New unknown reactions and interactions can, how unlikely they may be, have an profound impact on the system or our bacteria.

Overall, it can be concluded that even though there certainly are risks involved, the actual potential for harm seems minimal. The ideal solution to Panama disease would be a new resistant cultivar, but so far research in this direction has been slow and difficult. Compared to the current alternatives such as chemical treatment of the soil or the use of fungicides, which are known to severely disrupt the local environment, our system actually has a minimal footprint. However, as with all new technology, it is important to carefully assess the risks involved before wide-spread application and elaborate laboratory and field trials would be necessary to get more insight in this area.

Why our product?

First of all the alternatives have various drawbacks. Using toxins as farmers do now will not kill the spores of *Fusarium* and will eventually lead to fungicide resistance. Besides that fungicides are expensive and will massively disturb the ground ecology. Since natural breeding of bananas is extremely difficult and time consuming the other alternative is GMO bananas. The acceptance of GMO foods is low and GMO bananas can probably not be sold in Western markets such as Europe at the current time. Since the final product produced by the farmers who would use our bacteria is not actually GMO, acceptance would possibly be easier. Combined with the minimal ecological impact our product would have, it can be a real improvement compared to currently available options.

Future use and applications

With the rise of more highly engineered crops, whether created with traditional breeding or genetic modification, the inter-species diversity in crops grown commercially decreases. Even though it will not be as dramatic as is the case with bananas, crops will become less diverse and therefore weaker against threats they were not specifically selected for. Fusarium has been known to also infect other crops such as wheat, cucumbers and tomatoes. If successful, our system could rapidly be changed to work with other crops or even other fungus. Future applications could even include the production of plant growth promoters or increased nitrogen fixation in the soil. By combining the modularity and rapid modification time possible in bacteria but not plants, our system can be an efficient way to safeguard the crops of the future.

In summary

Our product uses a highly targeted way to combat *Fusarium* with a minimal disruption of the soil microbiome and environment, making it as eco-friendly as possible. Several safety measures will prevent the spread of modified genetic material and will kill the bacteria when its job is done, assuring the best possible safety. The bacteria can be grown and used very easy and cheaply, since the bacteria can be grown on cheap media and applied with methods and materials that are already present. Finally, our system will be highly modular and has the potential to be used for other crops *Fusarium* is known to infect, such as wheat, cucumbers and tomatoes, enabling wider usage to protect our food supply.